

pBOOST2-samIRF73

New DNA vaccine adjuvant of the pVAC plasmids expressing a super-activated IRF7/3 chimeric gene

Catalog # pbst2-samirf73

For research use only

Version 23E12-MM

PRODUCT INFORMATION

Content:

- 20 µg of lyophilized pBOOST2-samIRF73 plasmid expressing a mouse super-activated IRF7/3 chimeric gene
- 1 ml of Zeocin™ (100 mg/ml)

Shipping and storage:

Products are shipped at room temperature.

Lyophilized DNA is stable for 12 months when stored at -20°C.

Resuspended DNA is stable for 12 months when stored at -20°C. Avoid repeated freeze-thaw cycles.

Store Zeocin™ at 4 °C or at -20 °C. The expiry date is specified on the product label.

Quality control:

Plasmid construct has been confirmed by restriction analysis and sequencing.

Plasmid DNA was purified by ion exchange chromatography and lyophilized.

GENERAL PRODUCT USE

pBOOST2 plasmids were developed as genetic adjuvants for DNA vaccines to potentiate the immune response to a specific antigen. They feature different genes from the interferon regulatory factor family (IRF). IRFs are transcriptional activators for IFN- α , IFN- β and IFN-stimulated genes. In particular IRF-1, IRF-3 and IRF-7 act as direct transducers of virus-mediated signaling pathways activating IFN- α and IFN- β in infected cells. Recently, IRF-1, IRF-3 and IRF-7 were shown to be able to bias T cells towards type 1 or type 2 immune responses, leading to the activation of cytotoxic T cells and/or the production of antibodies. The method of plasmid DNA vaccine delivery is known to bias the immune response to a specific antigen towards a type 1 (T-cell) or type 2 (antibody) response¹. These biases can be further enhanced by the codelivery of IRFs to increase the efficacy of the vaccination^{2,3}.

PLASMID FEATURES

• **samIRF73** (super-activated mouse IRF7/3 chimeric gene)

IRF-3 and IRF-7 increase both Th1 T-cell and Th2 antibody responses by transactivating different target promoters². To exploit the biological features of both IRFs, a chimeric form of IRF-7 and IRF-3 was generated by combining the DNA binding specificity of IRF-7 with the strong transactivation capacity of super-activated IRF-3. IRF-7/3 chimera provides >10-fold greater induction of IFN- α and IFN- β promoters than super-activated IRF-3 alone³.

• **hEF1 / HTLV prom** is a composite promoter comprising the Elongation Factor-1 α (EF-1 α) core promoter⁴ and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat⁵. The EF-1 α promoter exhibits a strong activity and yields long lasting expression of a transgene *in vivo*. The R-U5' has been coupled to the EF-1 α core promoter to enhance stability of RNA.

• **SV40 pAn**: The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA.

• **Ori** is a minimal *E. coli* origin of replication with the same activity as the longer Ori.

• **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.

• **Sh-ΔCpG (Synthetic Zeocin® gene):** The *Sh ble* gene from *Streptallotheichus hindustanus* encodes a small protein that confers resistance to Zeocin™ by binding to the antibiotic. To reduce the amount of CpG motifs that may skew the raised antigen-specific immune response, pBOOST2 contains a CpG-free allele of the Zeo^R gene. All CpGs from the wild-type gene (50) were removed by synthesizing a new allele that contains no CpGs but encodes the exact same protein sequence.

References:

1. Robinson HL., 1999. DNA vaccines: basic mechanism and immune responses (Review). *Int J Mol Med*. 4(5):549-55.
2. Sasaki S. *et al.*, 2002. Regulation of DNA-raised immune responses by cotransfected interferon regulatory factors. *J Virol*. 76(13):6652-9.
3. Bramson JL. *et al.*, 2003. Super-activated interferon-regulatory factors can enhance plasmid immunization. *Vaccine*. 21(13-14):1363-70.
4. Kim, D.W. *et al.*, 1990. Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. *Gene* 2: 217-223.
5. Takebe, Y. *et al.*, 1988. R alpha promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. *Mol. Cell Biol*. 1: 466-472.

METHODS

Plasmid resuspension

Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1 µg/µl, resuspend the DNA in 20 µl of sterile H₂O. Store resuspended plasmid at -20 °C.

Plasmid amplification and cloning

Plasmid amplification and cloning can be performed in *E. coli* GT116 or in other commonly used laboratory *E. coli* strains, such as DH5α.

Zeocin™ usage

This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media and at 50-200 µg/ml to select Zeocin™-resistant mammalian cells.

TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873

InvivoGen USA (International): +1 (858) 457-5873

InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Hong Kong: +852 3622-3480

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Intramuscular inoculation

Plasmid DNA solution

- Prepare the vaccine plasmid solution by resuspending 10 μ g of the vaccine plasmid DNA in 50 μ l saline solution.
- Prepare the pBOOST2 solution by mixing 10 μ g of pBOOST2-samIRF73 and 90 μ g of the mock plasmid pBOOST2-null in 50 μ l saline solution for low dose, or 100 μ g of pBOOST2-samIRF73 in 50 μ l saline solution for high dose.
- Combine both solutions to obtain a total of 110 μ g DNA in 100 μ l saline solution.

Note: The quantities are per mouse.

Intramuscular injections

- Inoculate 6 to 8-week old female BALB/c mice with 100 μ l plasmid DNA solution (described above) into the quadriceps at 0 and 4 weeks.
- Collect sera and analyze for antibodies at 8 weeks.

Note: For more information see the article by Sasaki S. et al.¹

TECHNICAL SUPPORT

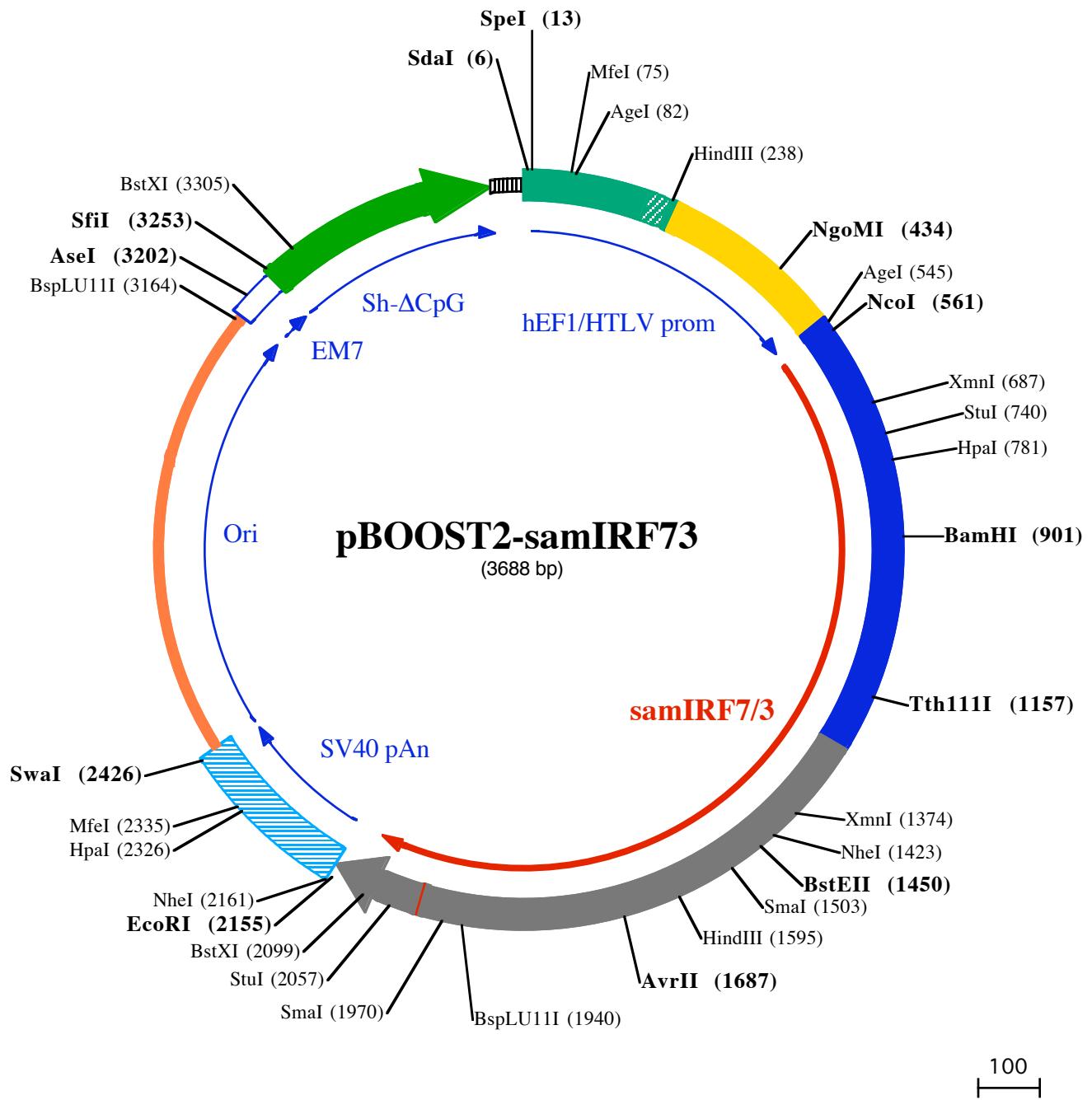
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SdaI (6) **SpeI (13)** MfeI (75) AgeI (82)
 1 CCTCGAGGGCCCACTAGTCAGTGGGCAGAGCGCACATGCCACAGTCCCAGAAGTTGGGGAGGGTCGCAATTGAACCGGTGCCTAGAGAAGGT
 101 GGCGCGGGTAAACTGGAAAGTGATGTCGTACTGGCTCGCCTTTCCCAGGGTGGGGAGAACGTATATAAGTCAGTAGTTGCCGTGACGT
 201 TCTTTTCGCAACGGTTGCGCCAGAACACAGCTGAAGCTTCGAGGGCTCGCATCTCCTCACCGCCCCGCCCTACCTGAGGCCATCCA
 301 CGCCGGTTAGTCGCTCTGCCGCTCCCGCTGGTGCCTCTGAACTGCGTCCGCCGTAGTAAGTTAAAGCTAGGTGAGACCGGGCTT
NgoMI (434)
 401 GTCCGGCGCTCCCTGGAGCCTACCTAGACTCAGCGGCTCCACGCTTGCCTGACCTGCTCAACTCTACGTCTTGTGTTCTGTTCT
AgeI (545) **NcoI (561)**
 501 GCGCCGTTACAGATCCAAGCTGTGACCGGCGCTACCTGAGATCACCGTAGGAGGGCACCATGGCTGAAGTGAGGGGGTCCAGCAGTGCTGTTGG
 13> 1 MetAl aVal aArgGl yVal Gl nArgVal LeuPheGl XmnI (687)
 601 AGACTGGCTATTGGGGAGGTAGCAGCGGCCAGTACGAGGGCTGAGTGGCTGAACGAGGCTGCACAGTCTCCGTAACCCTGGAAGCATTGCGT
 13> yAspTrpLeuLeuGl yGl uVal Ser Ser Gl yGl nTyrGl uGlyLeuGl nTrpLeuAsnGl uAl aArgThr Val PheArgVal ProTrpLysHisPheGl
 701 StuI (740) HpaI (781)
 47> CGTAGGGATCTGGATGAAGAAGATGCACAGATCTCAAGGGGGCTGTGGCCGAGGGGGCCACCTAGTGGGTTAACCTGCCACCCCCAGAGG
 801 ArgArgAspLeuAspGl uGl uAspAl aGl nI ePheLysAl aTrpAl aValAl aArgGl yArgTrpProProSer Gl yValAsnLeuProProProGl uA
 80> CTGAGGCTGCTGAGCGAAGAGAGCGAAGAGGCTGGAAGACCAACTCCGCTGTGCACTCCACAGCACAGGGCTTTATCTTGCGCCAACACAATTAGG
 80> IaGl uAl aAl aGl uArgArgGl uArgArgGl yTrpLysThrAsnPheArgCysAl aLeuHi sSer Thr Gl yArgPheI eLeuArgGl nAspAsnSer Gl
BamHI (901)
 901 GGATCCAGTTATCGCATAAGGTGTACGAACCTAGCCGGAGCTGGATCTACTGTGGGCCAGGCCACCGAAATAGGGAAGAAGTGAGCCTCAGCAAT
 113> yAspProAl AspProHi sLysValTyrGl uLeuSerArgGl uLeuGl ySer Thr Val Gl yProAl aThr Gl uAsnArgGl uGl uVal Ser LeuSerAsn
 1001 GCTCTCCCCACACAGGGTGTGCTCCAGGATCTTCTGCAAGAGAAATGCTGGCTCAAACCCCAGCCCTCTAGTGTGCCGGGAC
 147> Al aLeuProThr Gl nGl yVal Ser ProGl ySer PheLeuAl aArgGl uAsnAl aGl yLeuGl nThr ProSer ProLeuLeuSer SerAspAl aGl yAspL
Th111I (1157)
 1101 TCTTGCTTCAGGTTCTGAGTACAGCCACATACTGGAATCCGAGTCTGGGAGACCCCGTCCACCAGGCTCCTGCCAGGGAGAACCGTGTTA
 180> euLeuLeuGl nVal LeuGl nTyrSer HisI eLeuGl uSer Gl uSer Gl yAl aAspProVal ProProGl nAl aProGl yGl nGl uGl nAspArgVal Ty
 1201 CGAGGAACCTATGCAGCATGGCAGGTGGAAAGCTGTCCTCCAGTCCCTCCACTCCAGGAAACCTACCGAAGTTATTGATGCCCTGATCTGGGG
 213> rGl uGl uProTyrAl aAl aTrpGl nVal Gl uAl aValProSer ProLeuProHi sSer Gl nGl uAsnLeuProLeuPheAspGl yLeuI eLeuGl y
 1301 XmnI (1374)
 247> CCCCCTAAAGATGAGGGCTCTAGATCTGGTATTGTTCTGATCCTCTCAACAACTGCCAACGCCATGTAACACTCCCTAAACCTGCACCC
 1401 NheI (1423) **BstEII (1450)**
 280> AAGAAAATCCACTGAAGCAGTCTAGTGAGGAACAATGGAGTTGAGGTGACGCCCTTACCGAGGCCAGGTCTTCAGCAGACACTTTTG
 1501 HindIII (1595)
 313> InGl uAsnProLeuLysGl nLeuLeuAl aGl uGl nTrpGl uPheGl uVal Thr Al aPheTyrArgGl yArgGl nVal PheGl nGl nThr LeuPheCy
 1601 SmaI (1503)
 347> CCCCGGGGCCCTCGGGCTGGTGGGAGCACAGCTGACACTGCCCTGGCAGCCAGTCACCTGCCGATCTGAGGGTTCTGACGCCAACGCT
 1701 380> Val LysGl uTyrVal Gl yGl nVal LeuLysGl yLeuGl yAsnGl yLeuAl aLeuTrpGl nAl aGl yGl nCysLeuTrpAl aGl nArgLeuGl yHi sSer H
 1801 1701 ACGCTCTGGCTCTGGGGAGGAGCTGCTCCAGACAGTGGCGAGGGCTGATGGAGAGGCTCACAAGGACAAGGACGAGCCGTTGACCTCAG
 413> 380> iAl aPheTrpAl aLeuGl yGl uGl uLeuLeuProAspSer Gl yArgGl yProAspGl yGl nVal Hi sLysAspLysAspGl yAl aVal PheAspLeuAr
 1901 1801 GCCCTCGTGGCAGATCTGATTGCTTACATGGAGGACTCTCCACGCTACACTCTGGTTCTGACATGGGGAAATGTGCCAGGACAG
 447> 413> gProPheValAl aAspLeuI eAl aPheMetGl uGl ySer Gl yHi sSer ProArgTyrThr LeuTrpPheCysMetGl yGl uMetTrpProGl nAspGl n
BspLU11I (1940)
 1901 SmaI (1970)
 447> CCGTGGGCAAGAGGCTGTGATGGTCAAGGTTGTTCTACATGCTTAAGGAGCTGTTAGAGATGCCGGAGGGGAGCTCTACTGAAACCC
 2001 StuI (2057) **BstXI (2099)**
 480> 2001 TGGACTTCACATCGACAAACAGCCAGCTATCTCTTACCTCTGACCAAGTACAAGGCCACCTCCAGGACTTGGGAGGACATGGACTCCAGGCCAC
 513> 480> aAlAspLeuHi sI eAspAsnSer Gl nProI eSer LeuThr SerAspGl nTyrLysAl aTyrLeuGl nAspLeuVal Gl uAspMetAspPheGl nAl aTh
NeI (2161)
EcoRI (2155)
 2101 TGGAAATATCTGAGCCCCACTCAGCTGCTACCAATAAGCAGTTATGCCAGAACATTGCTGAGTTGGACA
 513> 513> rGl yAsnI e***
 2201 AACCACAACTAGAATGCACTGGAGAAAAAAATGCTTATTGTGAATTGTGATCTATTGTGAATTGTGATGCTATTGCTTATTGTGAA
 2301 HpaI (2326) MfeI (2335)
 2401 2301 CCATTATAAGCTGCAATAACAAAGTTAACAAACATTGCAATTCTTATGTTCAAGGTTCAAGGGGAGGTGGGAGGTTAAAGCAAGTAAAA
SwaI (2426)
 2501 2401 CCTCTACAAATGTTGAGATCCATTAAATGTTAACTAGCCATGACCAAAATCCCTAACGTGAGTTTCGTTCACTGAGCGTCAGACCCGTAG
 2601 2501 AAAAGATCAAAGGATCTCTGAGATCTTTCTGCGCTGATCTGCTGCAACACAAAAACCCACCGCTACCGGGTGGTTGCCGA
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 2801 2701 AAGAACTCTGAGACCGCTACATACCTGCTGTAATCCTGTTACAGTGGCTGCTGCCAGTGGCGATAAGTCGTCTACGGGTTGGACTCAA
 2901 2801 GACGATAGTTACCGATAAGGGCAGGGCTGAACGGGGTTCTGACACAGCCCAGCTGGAGGCAACGACCTACACCGAACTGAGATACT
 2901 2901 ACAGCGTGANCTAGAGAAAGGCCACGCTCCGAAGGGAGAAAGGGCGACAGGTATCCGTAAGCGCAGGGTGGAAACAGGAGAGCGCACGAGGGAG

3001 CTTCCAGGGGAAACGCCCTGGTATCTTATAGCCTGCGGTTGCCACCTCTGACTTGAGCGTCGATTTGTGATGCTCGCAGGGGGCGGAGCC

3101 TATGGAAAAACGCCAGCAACGCCCTTTACGGTCTGGCTTGCTGGCTTGCACATGTTCTAATTAAATTTCAAAAGTAGTTGACA

AseI (3202) SfiI (3253) BspLU1II (3164) BstXI (3305)

3201 ATTAATCATCGGCATAGTATATCGGCATAGTATAACGACTCACTATAAGGAGGCCATCATGGCCAAGTTGACCAGTGCTGCTCACAGCCA

3301 GGGATGTTGGCTGGAGCTGGAGTTCTGGACTGACAGGTTGGGTTCTCCAGAGATTTGTGGAGGATGACTTGCAGGTGGTCAGAGATGATGTCAC
14▶rAspValAlaGlyAlaValGluPheTrpThrAspArgLeuGlyPheSerArgAspPheValGluAspAspPheAlaGlyValValArgAspAspValTh
3401 CCTGTTCATCTCAGCAGTCCAGGACCAAGGTGGTGCCTGACAACACCCCTGGCTGGGTGAGAGGACTGGATGAGCTGATGCTGAGTGGAGTGAG
47▶rLeuPheIleSerAlaValGlnAspGlnValValProAspAsnThrLeuAlaTrpValTrpValIArgGlyLeuAspGluLeuTyrAlaGluTrpSerGlu
3501 GTGGTCTCACCAACTTCAGGGATGCCAGTGGCCCTGCATGACAGAGATTGGAGAGCAGCCCTGGGGAGAGAGTTGCCCTGAGAGACCCAGCAGGCA
81▶ValValSerThrAsnPheArgAspAlaSerGlyProAlaMetThrGluIleGlyGluGluProTrpGlyArgGluPheAlaLeuArgAspProAlaGlyA
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114▶snCysValHi sPheValAlaGluGluGlu nAsp***